Unveiling the Potential of Ruthenium Trichloride Clusters as Novel Therapeutic Agents in Combating Antifungal, Antibacterial, and Ant malarial Activities": A Comprehensive Research Study

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***Abstract*—Ruthenium trichloride complexes featuring η²-bound alkyne ligands are suggested as intermediates in the early stages of Ru and alkyne reactions. Our research has demonstrated that these unsaturated complexes, with the general formula [RuCl(η⁵-Cp) (η²-RC≡CR')], can be effectively stabilized by using a sterically demanding cyclopentadienyl ligand. Specifically, we have employed the η⁵-1-methoxy-2,4-ferrocenyl-3-neopentyl-cyclopentadienyl ligand (abbreviated as Cp). Additionally, we have found that trimerization of alkynes, demonstrating significant potential for applications in inorganic synthesis. In addition to our studies on the chemical properties and of ruthenium complexes, we have also conducted a thorough investigation of their biological activity. Specifically, we examined their potential as antifungal, antibacterial, and antimalarial agents. This comprehensive analysis aimed to explore the applicability of these complexes in medicinal chemistry, potentially leading to the development of new treatments for various infectious diseases.**

I. INTRODUCTION

The transition metal and cycloaddition of alkynes with triphenylphosphine substrates has gained significant attention as a powerful and efficient synthetic approach for the construction of structurally diverse and biologically relevant metal cluster [1]. This method offers several advantages, including high atom economy, regio- and stereoselectivity, and the ability to access complex molecular architectures in a streamlined manner. The involvement of transition metals facilitates bond activation, enabling the formation of new carbon metal and carbon-carbon bonds under mild reaction conditions [2-4]. In recent years, our research has been dedicated to investigating the reactivity of ferrocenyl and phenyl-substituted terminal and internal acetylenes in such cycloaddition reactions [5-6]. This reaction pathway allows for the controlled assembly of ruthenium atoms and chlorine ligands, guided by the presence of triphenyl phosgene as a versatile building block [7]. The resulting clusters possess intriguing structures that are dictated by the unique coordination preferences of ruthenium and the reactivity of the triphenyl phosgene moiety [8]. Polynuclear ruthenium clusters possess multiple metal centers that work cooperatively, enabling clusters and highly efficient biological processes. these multi-metallic coordination sites facilitate synergistic interactions that enhance biological activity, selectivity, and stability. These ruthenium clusters have found widespread use in a variety of applications as biocides. On account of their moderate to high level of biological activity [9]. This study aims to develop novel antimicrobial, antifungal, and antimalarial agents to address drug resistance and reduce the side effects associated with existing treatments. To achieve this, various alkynes (1,2,3) were utilized for the synthesis of ruthenium metal clusters. These metal clusters were then characterized using spectral techniques, including Fourier-transform infrared spectroscopy (FTIR). Additionally, their biological efficacy was evaluated through antifungal, antibacterial, antimalarial, and cytotoxicity assays.

II. MATERIALS AND METHODS

High-purity chemicals and solvents were used for biological activities and metal complex synthesis. Standard drying techniques were applied as needed. Methanol, ethanol, n-hexane, and phenyl acetylene were sourced from Merck; Ruthenium tri-chloride from Sigma Aldrich; biphenyl acetylene from BLD Chemicals; ethyl acetate from Fischer Chemicals; and ferrocenyl acetylene from RHD Chemicals.

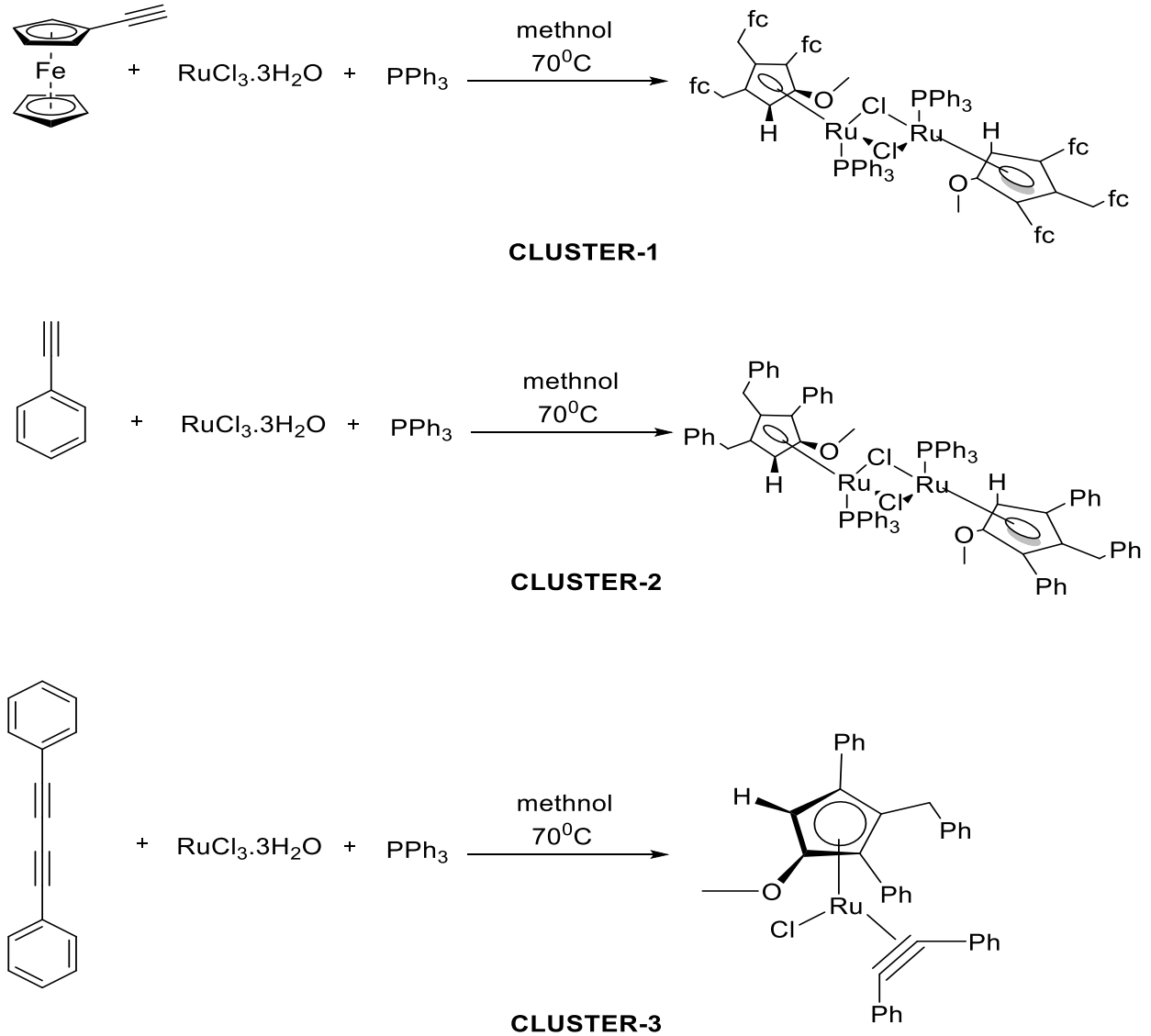

Fig-1 Reaction of the precursor $RuCl_3.3H_2O$ with the different ligands where L = ferrocenyl acetylene, phenyl acetylene, biphenyl acetylene [14-16].

SYNTHESIS

Cluster-1

Chemical Interaction Initiated by Combining 15 mg (0.0312 mmol) of Ruthenium Chloride [$RuCl_3.H_2O$], 10.65 mg (0.331 mmol) of Ferrocinyl acetylene, and 5 mg (0.2215) Triphenylphosphine (PPh_3) in methanol under Nitrogen Atmosphere at 100°C: Refluxing for 6 Hours and Analysis by Thin Layer Chromatography (TLC) Reveals Reactant Depletion and Product Formation. Further Purification of the Reaction Mixture was Achieved Through Chromatographic Separation on Silica Gel Using a hexane and ethyl acetate Solvent System.

Cluster-2

The reaction was initiated by carefully weighing and introducing 20 mg (0.0330 mmol) of Ruthenium

Chloride [$RuCl_3 \cdot H_2O$], 20 mg (0.1315 mmol) of phenyl acetylene, and 3 mg (0.1205 mmol) of Triphenylphosphine (PPh_3) into a reaction vessel containing methanol. To maintain an inert reaction environment and prevent unwanted side reactions, the setup was purged with nitrogen gas. The reaction mixture was then heated to 100°C and allowed to reflux for 6 hours, ensuring sufficient time for the transformation of reactants into the desired products. Upon completion of the reflux period, the progress of the reaction was monitored using thin-layer chromatography (TLC). To isolate and purify the obtained products, the crude reaction mixture was subjected to chromatographic separation using a silica gel column.

Cluster-3

The chemical interaction commenced by introducing 25 mg (0.0325 mmol) of the Ruthenium Chloride [$RuCl_3.H_2O$], 37 mg (0.243 mmol) of biphenyl acetylene and 5 mg (0.2215 mmol) Triphenylphosphine (PPh_3) in methanol under a nitrogen atmosphere, while maintaining a temperature of 100°C. The reaction mixture underwent reflux for a duration of 6 hours. Subsequent TLC analysis provided insights, revealing a significant depletion of the initial reactant and the emergence of new products. To further refine the reaction products, chromatographic separation of the crude reaction mixture was executed over silica gel, employing a solvent system comprising hexane and ethyl acetate.

III. RESULTS AND DISCUSSION

All ligands and their corresponding ruthenium metal clusters were identified using bands recorded through a Shimadzu FTIR spectrophotometer and a Bruker spectrometer (Vmax cm−1). The spectra were obtained using the total reflectance methodology.

To confirm the structural integrity of the ligands and their respective metal complexes, stretching vibrations of C=C, Ru-Cl, and Ru-P bonds were analyzed. The desired clusters and proposed structures aligned with the functional group stretching vibration data presented in Table 1.

For clusters 1, 2, and 3, the characteristic stretching bands for C=C appeared at 1408, 1491, 1408, 1493, 1410, and 1555 cm−1. In Cluster 1, the Cl-Ru-Cl (bridging chlorine) peak shifted to a lower frequency at 410 cm−1. Meanwhile, in Clusters 2 and 3, the bridging chlorine stretching frequencies were observed at 452 cm−1 and 425 cm−1, respectively. This shift towards lower frequencies indicates the bridging chlorine shift, which occurs due to electron donation from the ruthenium atom.

Table -1 FTIR spectroscopic data of metal complexes

S.No	Cluster	υC=C(cm^{-1})	υRu-Cl(cm^{-1})	υRu-P(cm^{-1})
1.	Cluster 1	1408	410	325-328
2.	Cluster 2	1408	452	326
3.	Cluster 3	1451	425	329

IV. BIOLOGICAL ACTIVITY

Biological activity involves a detailed assessment of both the beneficial and harmful effects that a compound may exert on living systems. This activity is determined not only by the molecule's intrinsic structure and physiological properties but also by its concentration and the length of time cells are exposed to it. Unlike pharmacological activity-which typically implies therapeutic benefits a compound is considered bioactive if it interacts with or influences any type of cellular tissue in the body. This concept has attracted significant interest across various fields, including ecology, biochemistry, and medical research.

Furthermore, the importance of inorganic chemistry in the realm of drug development has grown considerably. An increasing number of metal complexes are now used in both commercial and therapeutic applications, underscoring the critical need to understand their biological interactions. All the synthesized compounds were screened for biological activity in the micro care laboratory & Trc, Surat, Gujara**.**

Antimalarial Activity -

The in vitro antimalarial assay followed Rieckmann's micro assay protocol with slight modifications using *Plasmodium falciparum* 3D7 strain cultures. Parasites were maintained in RPMI 1640 medium with supplements and synchronized to the ring stage using 5% D-sorbitol.

An initial parasitemia of 0.8% to 1.5% at 3% hematocrit was set in 200 µl RPMI-1640 medium. JSB staining assessed parasitemia, with cultures maintained using 50% O+ RBCs. Test samples were prepared in DMSO (5 mg/ml), serially diluted, and added (20 µl) to wells, achieving final concentrations of 0.4–100 µg/ml in duplicate. Cultures were incubated at 37°C in a candle jar for 36–40 hours. Thin blood smears were stained with JSB, and microscopy assessed parasite maturation. The minimum inhibitory concentration (MIC) was the lowest concentration preventing schizont formation. Chloroquine, quinine served as the reference drug [21-23].

Antimalarial Activity evolution-

The antimalarial activity of the synthesized metal complexes was evaluated in vitro against *Plasmodium falciparum* using both a chloroquine-susceptible (CQS) strain (3D7) and a chloroquine-resistant (CQR) strain (W2). This dual-strain assessment provided insights into the efficacy of the tested compounds against both drug-sensitive and drug-resistant malaria parasites. The study aimed to determine whether the incorporation of ruthenium in these metal clusters could enhance antiplasmodial activity, particularly in comparison to standard antimalarial drugs. The results, summarized in Table 2, were compared against the reference drugs chloroquine and quinine, which served as positive controls. Remarkably, all three synthesized metal complexes—Cluster-1, Cluster-2, and Cluster-3—demonstrated significant antimalarial effects against the CQS strain (3D7), indicating their potential as effective inhibitors of *P. falciparum* proliferation. While all metal complexes exhibited considerable activity, Cluster-3 stood out with the highest potency, recording an IC_{50} value of 0.67 µg/mg, which suggests a strong inhibitory effect on parasite growth.

TABLE -2 ANTI-MALARIYAL ACTIVITY (PLASMODIUM FALCIPARUM)

S.No.	CLUSTER	MEAN IC_{50}
1	1	0.89 µg/ml
2	2	0.82 µg/ml
3	3	0.67 µg/ml
4	Standard drug chloroquine	200 µg/ml
5	Standard drug quinine	268 µg/ml

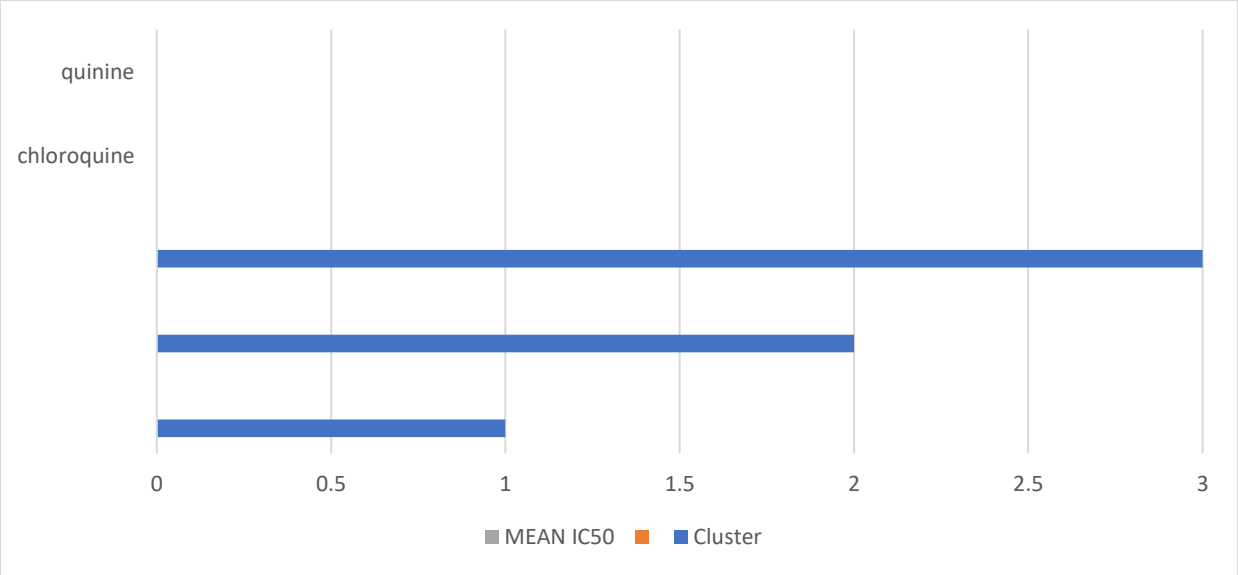

Fig.2 Anti-malarial activity

Antibacterial activity -

We evaluated the antibacterial efficacy of metal–ligand complexes against both gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria using the broth dilution method. Serial dilutions of the test organisms were prepared, and a control without antibiotics was subcultured by spreading a loopful of suitable medium over a portion of a plate before inoculation. After overnight incubation at 37°C, molten agar was dispensed into sterile petri dishes, cooled to 45°C, and inoculated with 0.6 mL of broth culture containing the test organism. Wells were created in the solidified agar using a sterile cork borer and filled with extracted agar plugs. Solutions of ligands and their metal complexes (1 mg/mL) were prepared, and 100 µL of each solution was transferred into the designated wells. Antibacterial activity was assessed by measuring the zone of inhibition in millimeters [10-13]

Antibacterial activity evaluation-

Metal complexes are screened for their antibacterial potential. Gram-negative strains (Escherichia coli and Staphylococcus aureus) and Gram-positive strain (Pseudomonas aeruginosa and S.Pyogneus) were used for antibacterial evaluation. The ref-erence drug used was gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin .The results of the antibacterial screening are summarized in Table 3. The ruthenium cluster-1 better result MTCC 443, MTCC 1688, bacterial cells but more minimal inhibition concentration MTCC 96 bacterial cells. Cluster-2 have a better result with MTCC 443 and MTCC 442 bacterial cells but more MIC with aeruginosa and aureus bacterial cells. In cluster-3 shows better result with e.coil, aureus but more MIC with aeruginosa and s.pyogenus.

Table-3 Antibacterial activity of metal Cluster

ANTIBACTERIAL ACTIVITY TABLE					
MINIMAL INHIBITION CONCENTRATION [MICROGRAM/ML]					
S.NO.	CLUSTER	E. COLI [MTCC 443]	P. AERUGINOSA [MTCC 1688]	S. AUREUS [MTCC 96]	S. PYOGENUS [MTCC 442]
1.	Cluster 1	100	100	75	200
2.	Cluster 2	200	400	500	200
3.	Cluster 3	75	150	75	100

TABLE-4 STANDARD DRUGS

S.No	DRUG	E. COLI MTCC 443	P. AERUGINOSA MTCC 1688	S. AUREUS MTCC 96	S. PYOGENUS MTCC 442
1		(MICROGRAMME/ML)			
2	GENTAMYCIN	.05	1	.25	0.5
3	AMPICILLIN	32		40	25
4	CHLORAMPHENICOL	50	50	50	25
5	CIPROFLOXACIN	25	25	50	50
6	NORFLOXACIN	10	10	10	10

Antifungal activity –

We evaluated the antifungal efficacy of test compounds against *Candida albicans*, *Candida glabrata*, and *Aspergillus Niger*. The fungi were cultured in Sabouraud Dextrose Broth at 28°C for 24 hours, then standardized to a 0.5 McFarland standard. Using a sterile cork borer, 6 mm diameter wells were created in the inoculated medium. Each well was filled with 50 µL of test samples at 1 mg/mL concentration. Nystatin served as the standard antifungal, and methanol as the negative control. Plates were incubated at 28°C for 48 hours after a one-hour pre-incubation at room temperature to facilitate diffusion. Antifungal activity was determined by measuring inhibition zones around the wells. The experiment was conducted in duplicate for thorough evaluation [10,16-17].

Antifungal activity evaluation

The antifungal activity of the synthesized metal complexes was evaluated using the broth dilution method, a widely accepted technique for determining the minimum inhibitory concentration (MIC) of antifungal agents. This method provides quantitative data on the effectiveness of the metal complexes against fungal strains by assessing their ability to inhibit fungal growth at various concentrations [18]. For this study, three fungal species were selected as test organisms: *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus*. These species were chosen due to their clinical relevance, as they are common causative agents of fungal infections in humans [19]. The antifungal activity of the ruthenium metal clusters was compared with standard antifungal drugs, Nystatin and Griseofulvin, which served as positive controls. The results of the antifungal evaluation, summarized in Table-5, revealed varying degrees of antifungal efficacy among the synthesized metal clusters. Among the tested complexes, Cluster-1 and Cluster-2 demonstrated superior antifungal activity against *Aspergillus niger*, indicating their strong inhibitory effects on this particular fungal strain. However, their activity against *Candida albicans* and *Aspergillus clavatus* was relatively lower, suggesting a strain-specific mechanism of action. In contrast, Cluster-3 exhibited enhanced antifungal activity against *Candida albicans*, surpassing its efficacy against *Aspergillus niger* and *Aspergillus clavatus*. These findings suggest that the structural and electronic properties of the metal clusters play a crucial role in determining their antifungal potency against different fungal species.

TABLE 5 -ANTIFUNGAL ACTIVITY OF METAL CLUSTER

S.NO.	CLUSTER	C.ALBICANS MTCC 227	A. NIGER MTCC 282	A. CLAVATUS MTCC 1323
1.	Cluster 1	500	250	400
2.	Cluster 2	300	200	400
3.	Cluster 3	250	500	400

TABLE 6 -THE STANDARD DRUGS (MINIMAL FUNGICIDAL CONCENTRATION)

DRUG	C.ALBICANS MTCC 227	A. NIGER MTCC 282	A. CLAVATUS MTCC 1323
NYSTATIN	100	100	100
GRESEOFULVIN	500	100	100

V. CONCLUSION

This research focused on the synthesis and biological evaluation of ruthenium trichloride metal clusters incorporating various phosphine (PPh_3) ligands. These metal clusters were synthesized using a systematic approach to ensure stability and reactivity, followed by thorough characterization through advanced spectral techniques [14]. The spectral characterization, including Fourier-transform infrared spectroscopy (FTIR), provided strong evidence supporting the proposed molecular structures and coordination environments of the synthesized ruthenium complexes [15].

After structural confirmation, the biological activity of these metal clusters was assessed through a series of bioassays to determine their antimicrobial, antifungal, and antimalarial potential. The antibacterial assays were conducted against various pathogenic bacterial strains, while the antifungal screening targeted clinically significant fungal species. Among the synthesized clusters, Cluster-2 exhibited the most potent antimicrobial activity, demonstrating a broad-spectrum effect against multiple bacterial and fungal strains. Specifically, antifungal assays revealed that Cluster-1 and Cluster-2 were more effective in inhibiting the growth of *Aspergillus niger* compared to *Candida albicans* and *Aspergillus clavatus*. In contrast, Cluster-3 displayed stronger antifungal activity against *Candida albicans*, surpassing its efficacy against *Aspergillus niger* and *Aspergillus clavatus*.

Furthermore, the synthesized ruthenium clusters were subjected to antimalarial screening against the chloroquine-sensitive (CQS) *Plasmodium falciparum* 3D7 strain. Notably, all three clusters (Cluster-1, Cluster-2, and Cluster-3) exhibited significant antimalarial activity, highlighting their potential as promising candidates for further development in antimalarial drug research [20-21]. The results suggest that ruthenium-based metal clusters, particularly those incorporating triphenyl phosphine ligands, could serve as effective pharmacological agents.

In conclusion, ruthenium metal clusters have demonstrated promising pharmacological potential.

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